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Eugenol dosages in the anesthetic induction of Amazonian Tambaqui under different temperatures

Dosagens de eugenol na indução anestésica do tambaqui da Amazônia sob diferentes temperaturas

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ABSTRACT

Ponds for fish farming in the Amazon Region have warm waters that require adjustments in the anesthetic management adopted for tropical fish species. The objective of this study was to evaluate the anesthetic behavior of tambaquis (*Colossoma macropomum*) submitted to two eugenol concentrations (26.5 and 53.0 mg.L^{-1}) at water temperatures of 27 and 31°C. In this study were used 20 fishes weighing 982 ± 199g in five replicates, individually sedated at predetermined eugenol concentrations in a temperature-controlled tank. The results showed that the fishes presented significant temporal differences ($p \leq 0.05$) in the period between deep anesthesia (00min:40sec) and recovery (02min:35sec) at the temperature of 31°C. The anesthetic effect at the dose of 53.0 mg.L^{-1}, regardless of temperature (27 or 31°C), was similar ($p \geq 0.05$) to the anesthetic effect at the water temperature of 31°C at the dose of 26.5 mg.L^{-1}. Therefore, it was more efficient ($p \leq 0.05$) for the shorter latency period for deep anesthesia. Within this context, temperature influenced the anesthetic effect and dose in tambaqui individuals.

Keywords: Behavior; *Colossoma macropomum*; deep anesthesia; recovery time; sedation

RESUMO

A temperatura da água de viveiros escavados das pisciculturas na região Amazônica são elevadas e demandam adequações no manejo com anestésicos adotado para as espécies de peixes tropicais. Objetivou-se avaliar o comportamento anestésico de tambaquis *(Colossoma macropomum)* submetidos a duas concentrações de eugenol (26,5 e 53,0 mg.L^{-1}) nas temperaturas da água de 27 e 31°C. Foram utilizados 20 peixes com peso corporal de 982 ± 199 g em cinco repetições, anestesiados individualmente nas concentrações de eugenol pré-determinadas em um aquário com temperatura controlada. Os resultados demonstraram que no período entre a anestesia profunda (00min:40seg) e a recuperação (02min:35seg), os peixes apresentaram diferenças significativas ($p \leq 0,05$) temporal sob a temperatura de 31°C. O efeito anestésico na concentração 53,0 mg.L^{-1}, independente da temperatura (27 ou 31°C) foi similar ($p \geq 0,05$) ao efeito do anestésico a temperatura da água de 31°C na dosagem de 26,5 mg.L^{-1}. Portanto mais eficientes ($p \leq 0,05$) pelo menor período de latência em que atinge a anestesia profunda. Dentro deste contexto a temperatura influenciou no efeito e na concentração anestésica dos indivíduos de tambaqui.

Palavras chave: Comportamento; *Colossoma macropomum*; anestesia profunda; tempo de recuperação; sedação

1 INTRODUCTION

The tambaqui (*Colossoma macropomum*) is the main native species farmed in Brazil, with great prominence in continental fish farming (Morais and O'sullivan, 2017). This species is easy to spawning using the hormonal induction in artificial environment, has good growth potential, and has high productivity under captivity conditions; in this way, the semi-intensive production of this fish species is growing throughout the country (Pedrosa Filho et al., 2016).

In the fish farming some routine practices involve the direct handling of animals, which expose them to stressors that affect its performance. Usually during the fish production the biometric measures of animals are needed in order to count it's biomass and feed rate procedures, thus, when handled, the tambaqui individuals become agitated, which causes them injuries and lose of scales, in consequence these animals become more susceptible to be affected by pathogens as fungus and bacteria's which can cause the fish death, also bring a high damage to the fish farming production system (Iguchi et al., 2003). In this sense, alternatives that can decrease stress for fish are important, thus it is frequently necessary to sedate or anaesthetize the fish before the handling procedures (Cunha et al., 2010; Rotili et al., 2012; Ke, et al., 2018).

Usually to choice an anesthetic to be used in the fish farming production, it will depends on the quality and the cost of the constituent, its availability in the market, its efficiency, the purpose of use, and the destination of the animal after the application of the drug, which are all characteristics met by eugenol compost (Roubach et al., 2005; Ke, et al., 2018).

Eugenol is a volatile aromatic compound present in clove (*Syzygium aromaticum*), which gives to this plant its remarkable aroma and flavor. In its leaves, it can represent approximately 95% of the extracted oil (Raina et al., 2001), which is also the main component of the oil from clove, varying from 70 to 85% (mazzafera, 2003).

Anesthetic substances associated with ideal temperature contribute to reduce hypermotility in the handling of animals, thus reducing the risk of accidents to the fish and to those who carry out the work (Moreira et al., 2015; Pattanasiri et al., 2016).

The Rondônia state, has been standing out as one of the largest tambaqui producers in the Northern region of Brazil (Ostrensky et al., 2008; Xavier, 2013). This region is considered one of the hottest regions in the country, the temperature has a strong influence on the anesthetic effect of eugenol in fish, which an increase in temperature can cause decrease in the period required for induction and anesthetic recovery (Hoskonem and Pirhonen, 2004). In this sense, this study evaluated the behavior of tambaqui to the anesthetic effect of eugenol in

different doses and water temperatures, aiming for test the hypothesis that the temperature did not influence in the tambaquis eugenol dosages.

2 MATERIAL AND METHODS

The study was developed at the Fishing and Aquaculture Laboratory of the Federal University of Rondônia, Brazil. We selected twenty juveniles of tambaquis (body weight average of 982 ± 199 g) from an excavated land pond, where the fish were forage by extruded commercial feed with 28% crude protein. The specimens under a 24 hour without food, were captured with a trawl (10 mm net), transported in fiberglass boxes to the laboratory, and placed in 100 liter isothermal boxes adapted with aerators for the supply of oxygen during the evaluation process. For the testing were used a completely randomized design in a factorial arrangement of two anesthetic dilution levels (26.5 and 53.0 $mg.L^{-1}$) and two temperatures (27°C and 31°C), with five replications.

The anesthetic used in the experiment was Eugenol® (Biodinâmica), soluble in alcohol (Affonso, 2012). The solution of the anesthetic, previously dissolved in the proportion of 1:20 eugenol:alcohol, was diluted in the concentrations of 26.5 and 53.0 $mg.L^{-1}$ of water in glass tanks equipped with oxygenators, until reaching 45 liters. The solution of the tanks, prepared individually by animal, was heated up until to the temperatures of 27 and 31°C, which were monitored by a digital thermometer. Therefore, was applied the same heating procedure in the water of the recovery tank, but without the anesthetic (control treatment). Each animal was sedated up to deep anesthesia. After that, the fish were carried out for the biometric measurement (toal weight and length) and subsequently placed in a recovery tanks, until they reached full recovery.

The behavioral characteristics of anesthetic induction evaluated were: light sedation (LSED), light anesthesia (LANE), and deep anesthesia (DANE). The recovery of the individuals was evaluated using the variables: placed on recovery tank (PORT), return of signals (RESI), recovery of balance (RECB), normal swimming (NSWI), and total recovery (Woody et al., 2002; Vidal et al., 2008). These procedures were performed through the approval of the Ethics Committee on the Use of Animals of the Federal University of Rondônia (CEUA # 018/2014).

In the statistical analysis, the means of the variables evaluated were submitted to ANOVA and compared by the Tukey test with $\alpha = 5\%$.

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3 RESULTS

The eugenol concentrations and water temperatures showed significant differences in the time of the anesthetic effect of tambaquis ($p \leq 0.05$). The eugenol concentrations were efficient in inducing all animals to deep anesthesia in up to 3 minutes (Figure 1A-C), but the hyperactivity signal preceded the calming effect in all animals. During anesthetic induction, the behavior of the fishes were followed by the behavioral pattern of hyperactivity during the first contact with the anesthetic, it were characterized by the agitated swimming against the walls of the tank, slow swimming, and the resting of the animal in the ventral dorsal position, in some cases, with slight inclination to one side and loss of reaction to external stimuli.

The ANOVA analysis shows that the anesthetic effect at the dose of 53.0 $mg.L^{-1}$ regardless of temperature (27 or 31°C) was similar ($p \geq 0.05$) to the anesthetic effect at the water temperature of 31°C at the dose of 26.5 $mg.L^{-1}$. Therefore, it's results were more efficient ($p \leq 0.05$) for the shorter latency period for deep anesthesia (DANE) than the lower dose (26.5 $mg.L^{-1}$) at the lower temperature (27°C) (Figure 1C).

For the milder anesthesia stages, given by the reactions of the animals evaluated in LSED and LANE, with some movements, were observed a difference ($p \leq 0.05$) between the dosages of 53.0 $mg.L^{-1}$ at 31°C and 26.5 $mg.L^{-1}$ at 27°C, with no differences ($p \geq 0.05$) between the other columns (Figure 1A and B).

The placement of fishes in the tank (PORT) with anesthetic-free in water was faster ($p \leq 0.05$) with higher temperature and the anesthetic dose, thus reflecting the efficiency of the anesthetic effect in biometry activities (Figure 1D).

In the tank with clear water, the fish presented a temporal difference ($p \leq 0.05$) for the beginning of the first signs of recovery (RESI) from the anesthetic effect between the treatment with the higher dosage of 53.0 $mg.L^{-1}$ at 31°C and lower dosage of 26.5 $mg.L^{-1}$ at 27°C, with 2:11 and 4:10 min respectively (Figure 1E). Despite the non-significant difference between the treatments with 53.0 $mg.L^{-1}$ regardless of temperature and 26.5 $mg.L^{-1}$ at 31°C in the recovery of balance (RECB), the results demonstrated a faster return trend for the fish submitted to the higher temperature (31°C) regardless of the eugenol dose (Figure 1F).

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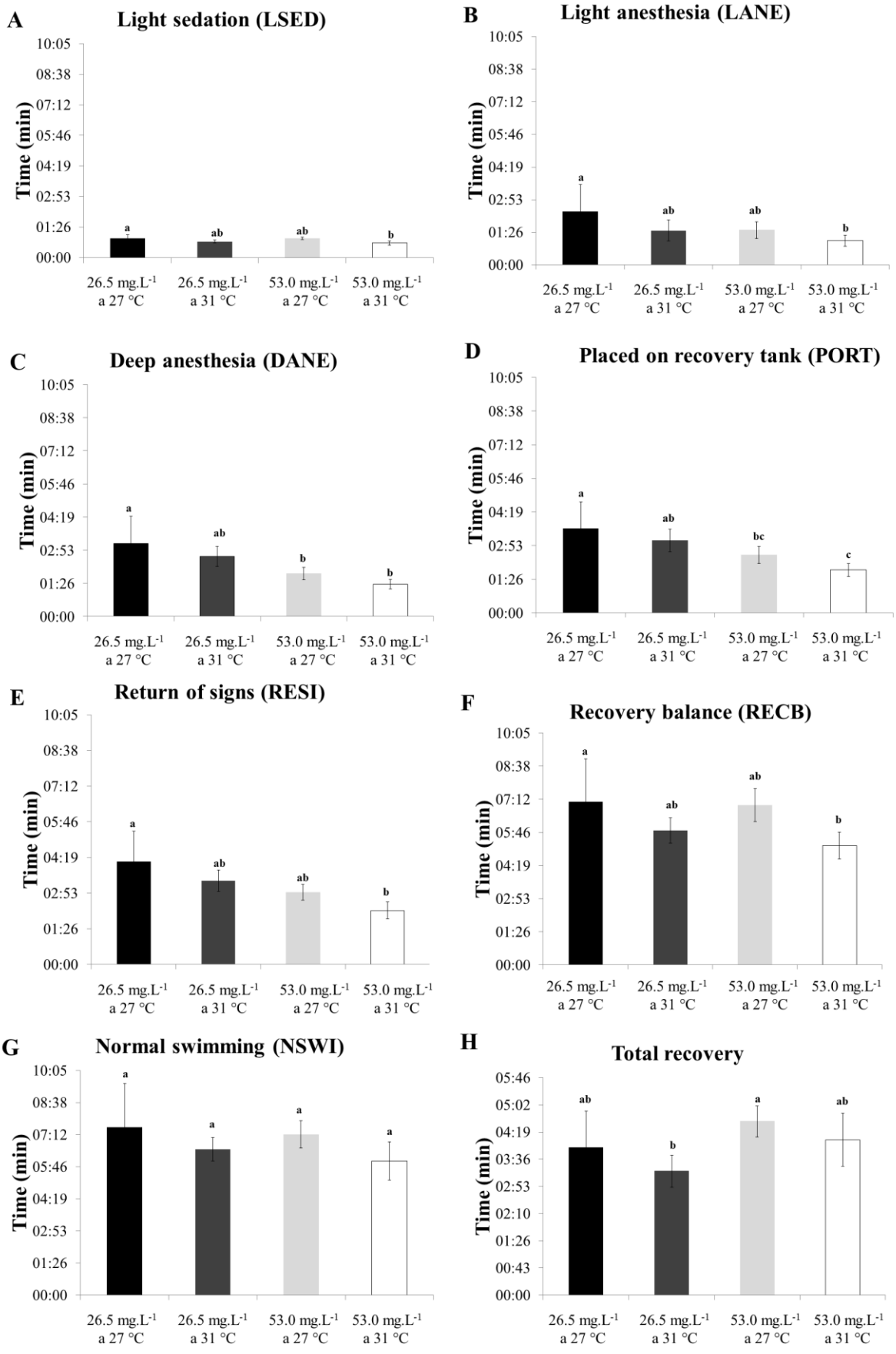

Figure 1 - Observation of the behavior of tambaquis to induction and recovery of anesthesia with eugenol. Columns with distinct letters differ from each other by the Tukey test at 5%.

When evaluating the sum of all recovery stages, excluding the time spent on induction and biometric management, we obtained the total recovery stages, which shows interesting results for the anesthetic effect ($p \geq 0.05$) at different doses and temperatures (Figure 1H). We can observe that the mean value for 26.5 $mg.L^{-1}$ at 31°C was the shortest period for total recovery from the anesthetic effect, while the lower temperature (27°C) at the higher dose (53.0 $mg.L^{-1}$) had a longer time for recovery from the anesthetic effect (3:17 vs 4:07 minutes).

4 DISCUSSION

The effect of the temperature on the anesthetic substance is related to the variation of fish body temperature, as this affects the speed of the biochemical reactions and raises the kinetic energy of the atoms and molecules facilitating the fish reactions (Baldisserotto, 2013).

Fishes have a body temperature that is similar to the environment temperature, which increases the metabolic rate of the animals and consequently the oxygen consumed, which is compensated by the greater number and range of respiratory movements, thus allowing more water to pass through the gills, as well as the substances dissolved in it (Zeni et al., 2016), also this pattern in the environment temperature may be influenced the different eugenol concentrations and water temperatures which influenced the time of the anesthetic effect in tambaqui fish for the present study.

In cases with higher water temperatures, approximately 31°C, the fish farmer can reduce the amount of anesthetic used in the management of animals, as the efficiency of the double of the dose were the same in this study. The dose of eugenol related to 26.5 $mg.L^{-1}$ at 31°C was so efficient as the dose of 53.0 $mg.L^{-1}$ at 31°C.

In the first contact with the immersion bath in the eugenol solution, the fish showed a pattern of hyperactivity. The same pattern of euphoria and rapid movement in the tank has been observed by INOUE et al. (2011) and also in other fish species that were induced to deep anesthesia using eugenol, such as *Brycon cephalus*, *Oreochromis niloticus* (Vidal, 2008) and pacu (*Piaractus mesopotamicus*) (Rotili et al., 2012; Sanchez et al., 2014).

Some authors point out that the recovery time of the fish is influenced by the time of exposure to the drug and the temperature, regardless of the dose used (Rotili et al., 2012; Sanchez et al., 2014). A good anesthetic induction should have a short latency time (1 to 3

min) with recovery that does not exceed 5 min (Marking and Meyer, 1985; Delbom and Ranzani Paiva, 2012; Duarte et al., 2015). In the present study, the exposure of the tambaquis to eugenol remained within this range, which is in agreement with studies with anesthetics. We highlight that this study did not present the mortalities of the fish after recovery.

Although the results did not exhibit statistically significant differences between the concentrations and temperatures values tested when analyzing normal swimming behavior (NSWI) (Figure 1G), we can observe a trend of fast response regardless of the concentration at the higher temperature tested (31°C). Therefore, we can observe higher anesthetic efficiency at higher temperatures than in the applied doses.

When we compared the lower concentration of eugenol at the higher temperature and the higher concentration of this solution at the lower temperature tested, we observed a shorter period for total recovery in the lower concentration with higher temperature (26.5 $mg.L^{-1}$ at 31°C) (figure 1 H). This pattern can be explained by the efficiency in the fish metabolism of the anesthetic in the fish organisms, when submitted to a higher temperature and lower dose of the anesthetic; it was also similar ($p \leq 0.05$) to the period spent in the higher temperature at 53 $mg.L^{-1}$, given the higher metabolic activity and lower residual anesthetic effect in the individuals, even though the dose was double the amount, and similar to the lower temperature at 26.5 $mg.L^{-1}$, with fast fish recovery related to the lower Eugenol dose. In contrast, low temperatures, even with a higher dose of the anesthetic (53 $mg.L^{-1}$ at 27°C) may hinder the metabolism of the anesthetic in the animal and delay the recovery time. In addition to these effects involving fish metabolism and behavior, anesthetics have another factor of great relevance, since most of them are absorbed in the animal tissues, which can accumulate residues (Marking and Meyer, 1985) but also, eliminated with purification. The purification rate depends on the type of drug used, target species, concentration, and route of administration, which can take a few hours to several weeks (Delbom and Ranzani Paiva, 2012). Even though anesthetic residues present in fish are not harmful to human consumption (Ke et al., 2018), they may interfere in the natural flavor of the fish (Cunha et al., 2010).

At higher temperatures, the metabolic rate of fishes is higher and induction to anesthesia is consequently faster. Ke et al. (2018) have observed eugenol residues ranging from 3.1 to 30.7 $\mu g.kg^{-1}$ in the higher concentration of the compound in fish from colder seawater compared to hotter freshwater, 19.0% vs 4.7% respectively. In the present study, the results show that the temperature of the pounds water that supplied the tambaqui farms, had varied between 27.0 to 39.7°C, with a mean value of the ponds around 29.0°C. We highlight that the

water temperatures of shallow ponds or with a high fish density commonly reach values higher than 29°C in the study area. The results also shows that the use of anesthesia by the immersion method, using Eugenol solutions, during tambaqui management, proved that is feasible for the animals in farming procedure, and can be safety for the fish and manipulators during handling.

5 CONCLUSION

The eugenol dose of 26.5 $mg.L^{-1}$ at a temperature of 31°C was the most indicated for routine practice in the fish farms, due to its efficiency of deep anesthesia signs in fish individuals, which was as well similar to the dose of 53 $mg.L^{-1}$, that presented effectiveness in fish recovery time. In summary, the temperature influences the anesthetic effects on the eugenol dosage for tambaqui fish, which indicates an apparently less stress for the animals during the handling procedures.

REFERENCES

AFFONSO, R. S.; RENNÓ, M. N.; SLANA, G. B. C. A.; FRANCA T. C. C. [2017]. Aspectos Químicos e Biológicos do Óleo Essencial de Cravo da Índia. **Revista Virtual de Química**, v.4, n.2, p.146-161, 2012. Available at <http://rvq-sub.sbq.org.br/index.php/rvq/article/view/254>. Accessed in November 09th, 2017. doi: 10.5935/1984-6835.20120012**.**

BALDISSEROTTO, B. **Fisiologia de peixes aplicada à piscicultura.** Santa Maria: [s.n], 2013. 350 p.

BOIJINK, C. L.; MIRANDA W. S. DA C.; CHAGAS, E. C.; DAIRIKI, J. K.; INOUE L. A. K. A. [2015], Anthelmintic activity of eugenol in tambaquis with monogenean gill infection. **Aquaculture**, v.438, n.1, p.138-140, 2015. Available at <https://www.sciencedirect.com/science/article/pii/S0044848615000174/> Accessed in February 01th, 2018 doi: https://doi.org/10.1016/j.aquaculture.2015.01.014.

CUNHA, M. A.; ZEPPENFELD, C. C.; GARCIA, L. O.; LORO, V. L.; FONSECA, M. B.; EMANUELLI, T.; VEECK, A. P. L.; COPATTI, C. E.; BALDISSEROTTO, B. [2010]. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory

analysis of fleet. *Ciência Rural*, v.40, n.10, p.2107-2114, 2010. Available at <http://dx.doi.org/10.1590/S0103-84782010005000154>. Accessed in January 28th, 2018. doi: 10.1590/S0103-84782010005000154.

DUARTE, J. S.; HONORATO, C. A.; SANTOS, T. R. [2015]. Tempo de indução e recuperação da anestesia do eugenol para beta (*Bettas plendes*). **Revista Brasileira de Ciências Veterinária**, v.22, n.3-4, p.176-179, 2015. Available at <http://www.rbcv.uff.br/rbcv/article/viewFile/753/1283>. Accessed in February 25th, 2018. doi: 10.4322/rbcv.2016.010.

GONÇALVES, A. F. N.; SANTOS, E. C. C.; FERNANDES, J. B. K.; TAKAHASHI, L. S. [2008]. Mentol e eugenol como substitutos da benzocaína na indução anestésica de juvenis de pacu. **Acta Scientiarum Animal Sciences**, v.30, n.3, p.339-344. 2008. Available at <http://periodicos.uem.br/ojs/index.php/ActaSciAnimSci/article/view/1081/1081>. Accessed in October 5th, 2017. doi: 10.4025/actascianimsci.v30i3.1081.

GUERETZ, J. S.; SOMENSI, C. A.; SOUZA, A. P. S. [2017]. Evaluation of eugenol toxicity in bioassays with test-organisms. **Ciência Rural**, v.47, n.12, 2017. Available at <http://www.scielo.br/scielo.php?pid=S0103-84782017001200403&script=sci_arttext>. Accessed in February 01th, 2018. doi: 10.1590/0103-8478cr20170194.

IGUCHI, K.; OGAWA, K.; NAGAE, M.; ITO, F. [2003]. The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). Aquaculture, v. 202, p.515-523, 2003.

KE, C.; LIU, Q.; LI, L.; CHEN, J.; ZHAO, C.; XU, J.; HUANG, K.; MENGSONG, M.; LI, L. [2018]. Residual levels and risk assessment of eugenol and its isomers in fish from China markets. **Aquaculture**, v.484, n.1, p 338-342, 2018. Available at <https://www.sciencedirect.com/science/article/abs/pii/S0044848617308463>. Accessed in February 25th, 2018. doi: 10.1016/j.aquaculture.2017.07.034.

MARKING, L. L.; MEYER, F. P. [1985]. Are better anesthetics needed in fisheries? **Fisheries**, v.10, n.6, p.2-5, 1985. Available at <http://onlinelibrary.wiley.com/doi/10.1577/1548-

8446(1985)010%3C0002:ABANIF%3E2.0.CO;2/abstract>. Accessed in February 25th, 2018. doi: 10.1577/1548-8446(1985)010<0002:ABANIF>2.0.CO;2.

MAZZAFERA, P. M. [2003]. Efeito alelopático do extrato alcoólico do cravo-da-índia e eugenol. **Revista Brasileira Botânica**, v.26, n.2, p.231-238, 2003. Available at <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-84042003000200011>. Accessed in February 8th, 2018. doi: 10.1590/S0100-84042003000200011.

MORAIS, I. S.; O'SULLIVAN, F. L. A. [2017]. Biologia, habitat e cultivo do tambaqui *Collosoma macropomum* (CUVIER, 1816). **Scientia Amazonia**, v.6, n.1, p.81-93, 2017. Available at <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/153405/1/v6-n1-81-93-2017.pdf>. Accessed in October 26th, 2017.

MOREIRA, A. G. L.; MOREIRA, A. G. L.; COELHO, A. A. C.; ALBUQUERQUE, L. F. G.; MOREIRA, R. T.; FARIAS, W. R. L. [2015]. Eugenol effect as a mitigate agent of stress in transport of Nile tilapia juveniles. **Pesquisa Veterinária Brasileira**, v.35, n.11, p.893-898, 2015. Available at <http://dx.doi.org/10.1590/S0100-736X2015001100004>. Accessed in September 27th, 2016. doi: 10.1590/S0100-736X2015001100004.

PATTANASIRI, T.; TAPARHUDEE, W.; SUPPAKUL, P. [2017]. Acute toxicity and anaesthetic effect of clove oil and eugenol on Siamese fighting fish, *Betta splendens*. **Aquaculture International**, v.25, n.1, p.163-175, 2017. Available at http://link.springer.com/article/10.1007/s10499-016-0020-2/doi: 10.1007/s10499-016-0020-2. Accessed in September 27th, 2016. doi: 10.1007/s10499-016-0020-2.

PEDROZA FILHO, M. X.; RODRIGUES, A. P. O.; REZENDE, F. P. [2016]. Dinâmica da produção de tambaqui e demais peixe redondos no Brasil. **CNA Brasil Ativos aquicultura**, v.2, n.7, p.1-5, 2016. Available at https://www.embrapa.br/pesca-e-aquicultura/busca-de-publicacoes//publicacao/1041302/dinamica-da-producao-de-tambaqui-e-demais-peixes-redondos-no-brasil. Accessed in October 26th, 2017.

RAINA, V. K.; SRIVASTAVA, S. K.; AGGARWAL, K. K.; SYAMASUNDAR, K. V.; KUMAR, S. [2001]. Essential oil composition of *Syzygium aromaticum* leaf from little Andaman, India. **Flavour Fragrance Journal**, v.16, p.334–336, 2001. Available at

<http://onlinelibrary.wiley.com/doi/10.1002/ffj.1005/full>. Accessed in September 27th, 2016. doi: 10.1002/ffj.1005.

ROTILI, D. A.; DEVENS, M. A.; DIEMER, O.; LORENZ, E. K.; LAZZARI, R.; BOSCOLO, W. R. [2012]. Eugenol as anesthetic for *Piaractus mesopotamicus*. **Pesquisa Agropecuária Tropical**, v.42, n.3, p.288-294, 2012. Available at <http://www.scielo.br/pdf/pat/v42n3/a06v42n3.pdf>. Accessed in September 27th, 2016. doi: 10.1590/S1983-40632012000300013.

ROUBACH, R.; Gomes, L. C.; Fonseca, F. A. L.; Val, A. L. [2005]. Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). **Aquaculture Research**, v.36, n.11, p.1056-1061, 2005. Available at <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2109.2005.01319.x/abstract>. Accessed in October 25th, 2015. doi: 10.1111/j.1365-2109.2005.01319.x.

SANCHEZ, M. S. S.; Rodrigues, R. A.; NUNES A. L.; Oliveira, A. M. da S.; Fantini, L. E.; Campos, C. M. de [2014]. Efeito do mentol e eugenol sobre respostas fisiológicas do pacu *Piaractus mesopotamicus*. **Semina: Ciências Agrárias**, v.35, n.4, 2014. Available at <http://www.redalyc.org/html/4457/445744143045/index.html>. Accessed in November 25th, 2017. doi: 10.5433/1679-0359.2014v35n4Suplp2799.

VIDAL, L. V. O.; ALBINATI, R. C. B.; ALMEIDA, T. R. DE; SANTOS, G. B. [2008]. Eugenol as an anesthetic for Nile tilapia. *Pesquisa Agropecuária Brasileira,* v.43, n.8, p.1069-1074, 2008. Available at <http://dx.doi.org/10.1590/S0100-204X2008000800017>. Accessed in January 17th, 2017. doi: 10.1590/S0100-204X2008000800017.

WOODY, C. A.; NELSON, J.; RAMSTAD, K. [2002]. Clove oil as an anaesthetic for adult sockeye salmon: field trials**. Journal of Fish Biology**, v.60, p.340-347, 2002. Available at < http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.2002.tb00284.x/abstract>. Accessed in January 8th, 2016. doi: 10.1111/j.1095-8649.2002.tb00284.x.

ZENI, T. O.; OSTRENSKY, A.; WESTPHAL, G. G. C. [2016]. Respostas adaptativas de peixes a alterações ambientais de temperatura e de oxigênio dissolvido. **Archives of Veterinary Science**, v.21, n.3, p.01-16, 2016. Available at

<http://revistas.ufpr.br/veterinary/article/view/40165>. Accessed in February 25th, 2018. doi: 10.5380/avs.v21i3.40165.